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TREASURY DEPARTMENT.

Public Health and Marine-Hospital Service of the United States.

WALTER WYMAN, Surgeon-General.

HYGIENIC LABORATORY.—BULLETIN No. 19.

October, 1904.

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A METHOD FOR INOCULATING ANIMALS WITH PRECISE AMOUNTS.

BY

M. J. ROSENAU,
DIRECTOR OF THE HYGIENIC LABORATORY.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1904.

NOTICE TO LIBRARIANS AND BIBLIOGRAPHERS CONCERNING THE SERIAL PUBLICATIONS OF THIS LABORATORY.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress, March 3, 1901.

The following *bulletins* [Bulls. Nos. 1–7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

No. 1.—Preliminary note on the viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

No. 4.—Viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

No. 6.—Disinfection against mosquitoes with formaldehyd and sulphur dioxid. By M. J. Rosenau.

No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

By act of Congress, approved July 1, 1902, the name of the "United States Marine-Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the Service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition March, 1904.)

No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

No. 11.—Experimental investigation of *Trypanosoma lewisi*. By Edward Francis.

No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

No. 13.—A statistical study of the intestinal parasites of 500 white male patients at the United States Government Hospital for the Insane; by Philip E. Garrison, Brayton H. Ransom, and Earle C. Stevenson. A parasitic roundworm (*Agamomermis culicis* n. g., n. sp.) in American mosquitoes (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis;* by Ch. Wardell Stiles.

No. 14.—Spotted fever (tick fever) of the Rocky Mountains; a new disease. By John F. Anderson.

No. 15.—Inefficiency of ferrous sulphate as an antiseptic and germicide. By Allan J. McLaughlin.

No. 16.—The antiseptic and germicidal properties of glycerin. By M. J. Rosenau.

No. 17.—Illustrated key to the trematode parasites of man. By Ch. Wardell Stiles.

No. 18.—An account of the tapeworms of the genus *Hymenolepis* parasitic in man, including reports of several new cases of the dwarf tapeworm (*H. nana*) in the United States. By Brayton H. Ransom.

(Continued on third page of cover.)

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WALTER WYMAN, *Surgeon-General.*
United States Public Health and Marine-Hospital Service.

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A METHOD FOR INOCULATING ANIMALS WITH PRECISE AMOUNTS.

By M. J. Rosenau, Director Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service.

The need has long been felt in special lines of research work for a good method of inoculating animals with precise amounts. In the ordinary laboratory work great exactness in this regard is not an important matter, but in standardizing diphtheria antitoxins, and in the physiological tests of some drugs, and in certain lines of physiological chemistry, the greatest precision is essential.

With the ordinary methods in use in most laboratories, there is an unavoidable loss, resulting in an error of from 1 to 8 per centum. The usual method consists of first measuring the dilution, or the substance, into a graduate or other suitable vessel, and then drawing it up into the syringe, from which it is inoculated into the animal.

There are two principal sources of error in this practice, (1) the loss in the glass and (2) the loss in the syringe.

The loss in the glass.—If the fluid is drawn up into the syringe from a glass container a certain amount remains behind. In a series of 18 careful weighings this loss was found to average about one-half of 1 per cent, using 4 c.c. of fluid.

The loss from this source was determined by weighing a chemically clean conical graduate, then adding 4 c.c. of distilled water, taking care not to wet more surface than necessary, and again weighing to determine the amount of water. As much of the water as possible is drawn up into the syringe, tilting the graduate so as to get the last drop that drains to the bottom. The graduate is again quickly weighed before evaporation takes place to any appreciable extent. The difference between the weight of the wet glass and the dry glass of course gives the loss in the glass. The average loss in 4 c.c. was found to be 0.0192; that is, nearly two one-hundredths of a cubic centimeter, or about one-half of 1 per cent (0.48 per cent) of the amount of water used. Of course when less than 4 c.c. of fluid is used the percentage of loss increases in proportion.

The variation in the loss is shown in the following figures:

	Loss in graduate, using 4 c. c. of water.
(1)	0. 0366
(2)	. 0032
(3)	. 0095
(4)	. 0098
(5)	. 0175
(6)	. 0176
(7)	. 0112
(8)	. 0124
(9)	. 0301
(10)	. 0077
(11)	. 0098
(12)	. 0156
(13)	. 0220
(14)	. 0313
(15)	. 0636
(16)	. 0247
(17)	. 0121
(18)	. 0107
	18) . 3454
	. 0192 average.

Notice that sometimes the loss amounts to six hundredths of a cubic centimeter, that is as much as 1.5 per cent.

The loss in the syringe.—The water that was drawn up into the syringe is now emptied into another carefully weighed graduate and the added weight determined. The difference gives the total loss, and the difference between the total loss and the loss in the graduate gives the loss in the syringe.

As much as possible of the contents of the syringe is pressed out by means of an air cushion behind the column of water. However, as will be seen by the figures, an appreciable amount remains behind; especially if the syringe has a shoulder or a poor packing presenting a large wetted surface.

The following figures give the result of eighteen weighings to determine the loss in the syringe:

	Loss in the syringe.	
(1)	0. 0219	A special syringe, "Sub Q" joint, no shoulder.
(2)	. 0097	A special syringe, "Sub Q" joint, no shoulder.
(3)	. 0295	A special syringe, "Sub Q" joint, no shoulder.
(4)	. 0307	A special syringe, "Sub Q" joint, no shoulder.
(5)	. 0147	A special syringe, "Sub Q" joint, no shoulder.

	Loss in the syringe.	
(6)	0.0272	A special syringe, "Sub Q" joint, no shoulder.
(7)	.0224	A special syringe, "Sub Q" joint, no shoulder.
(8)	.0315	A special syringe, "Sub Q" joint, no shoulder.
(9)	.0119	A special syringe, "Sub Q" joint, no shoulder.
(10)	.0232	A special syringe, "Sub Q" joint, no shoulder.
(11)	.0401	A special syringe, "Sub Q" joint, no shoulder.
(12)	.3363	Colin syringe with old packing.
(13)	.0871	Colin syringe with old packing.
(14)	.0276	New Colin syringe.
(15)	.0855	New Colin syringe.
(16)	.0747	New Colin syringe.
(17)	.1153	New Colin syringe.
(18)	.0969	New Colin syringe.
	18) 1.0862	
	.0603	Average loss in the syringe.

More is lost in the syringe than in the graduate, and the two together make an appreciable amount, in one instance (weighing No. 12) to more than 8 per cent of the amount of fluid used.

The new method.—To avoid these errors, the amount of fluid which the animal is to receive is now measured directly into the barrel of the syringe, thereby totally eliminating the loss in the graduate; and the fluid remaining in the syringe after it is emptied, is washed out with sterile salt solution or other suitable fluid which is then injected into the animal, so that the loss, if any, is not appreciable.

The syringe used for carrying out this technique is a modification of the old Koch syringe. The glass barrels for the syringe were made for me by the Randall-Faichney Company, of Boston, who mold a thread in the glass on the end of the barrel so that the needle screws directly upon the glass. This is a distinct advantage and has many points to recommend it over the old slip joint, particularly in that the joint is very tight, and no amount of pressure can force the needle from its bearings.

The bulb was made for me by Lenz & Lossau, of Washington, D. C., and as will be seen by reference to the illustration, differs from the Koch pattern in that there is no stopcock. The soft rubber stopper fits into the syringe like the cork in a bottle (see fig. 2) and is adjusted more readily than the ground glass and metal cap of the Koch syringe.

Note that the barrel tapers gradually to the needle so that the last drop will readily run out. The top has a slight flange which is handy in holding the syringe in use.

These slight modifications in the construction of the syringe are intended to facilitate its use as well as to increase its accuracy. The way of using these syringes differs so manifestly from any known to me that I will describe it in detail, for the precision depends as much upon the method of using them as upon their construction.

The glass barrels are cleaned in a saturated solution of chromic acid in sulphuric acid which leaves them chemically clean and prevents water or other fluids sticking to the glass in large drops. The acid is thoroughly washed out with tap water and then flushed in distilled water, allowed to dry and sterilized by dry heat. Clean glass allows a free flow, diminishing the amount of water which adheres to the surface, and thereby helps to thoroughly empty the contents.

The needles are sterilized separately by the usual method of boiling in a 1 per cent sodium carbonate solution.

The needle is now screwed on the barrel of the syringe and the joint tested by drawing some sterile salt solution in and out several times. If the joint is tight and the needle pervious, the outside is dried with a little piece of sterile gauze, and the needle is now plunged into a jar of sterilized albolene. The albolene acts as a temporary plug, preventing any of the fluid which is placed in the syringe from escaping until it is injected into the animal.

The necessary number of syringes are prepared, one for each animal. In testing diphtheria toxin and antitoxin we sometimes have a battery of ten, twenty, or thirty syringes thus prepared, arranged on the rack as shown in the accompanying diagram. (Fig. 1.)

The amount of fluid desired to inject into the animal is now carefully measured directly into the barrel of the syringe. This is injected into the animal, and then without withdrawing the needle the bulb is removed; a few cubic centimeters of salt solution are quickly blown into the barrel, washing down the sides, and this in turn is injected into the animal.

The syringes can not be turned over, as is the case with a piston syringe. It is not necessary, however, to hold them upright, as might at first be imagined. They may be tilted to a greater angle than 45° without danger of the fluid running out.

The rack in which the syringes are shown (fig. 1) is made of wood and has several conveniences worth noting. The bottom shelf is of glass. The least drop through the needle or from a leaky joint is plainly seen on the glass, which acts as a telltale against this possible error.

I also write upon the glass plate the amount each syringe is to receive, which helps to avoid mistakes and facilitates the work.

Each glass barrel is numbered with the number of the experiment or of the animal to receive the injection. This is very convenient and avoids confusion.

The syringes are prepared and filled in a special room where the exact measuring is done, and the rack is then found handy to carry the syringes to the animal room, where the work of injection is carried on.

In standardizing diphtheria antitoxin we always inject a total of 4 c. c. of fluid, allowing only one washing, but when the total amount of fluid injected is immaterial, especially in larger animals, the syringe may be washed out several times with a neutral fluid.

In using this method in standardizing diphtheria antitoxin it is important to shake and roll the syringe so as to obtain an intimate mixture of the toxin with the serum.

In working with definite weights of solids the solution may be made in the barrel of the syringe, so that the method is applicable in any sort of work where it is important to inoculate animals with precise amounts.

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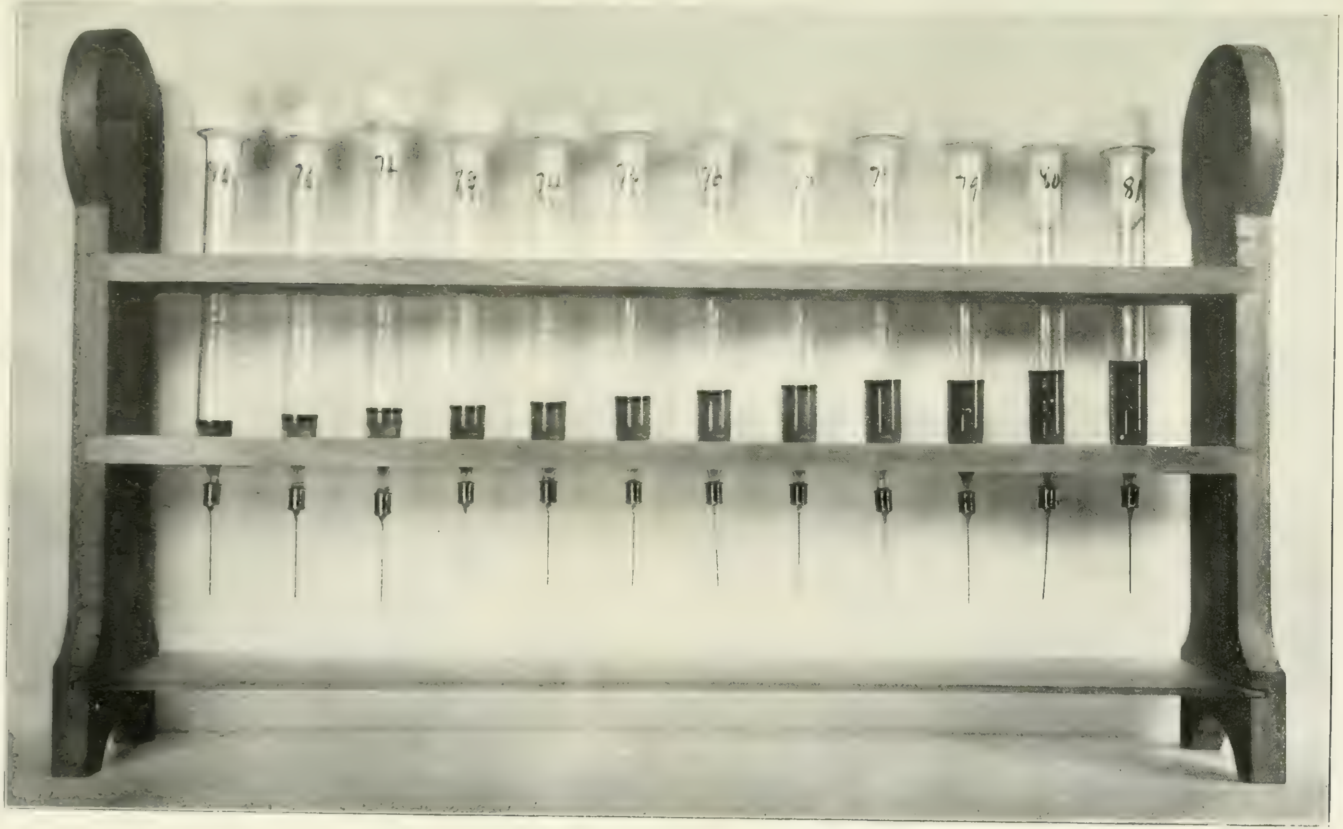

FIG. 1.—A BATTERY OF 12 SYRINGE BARRELS READY FOR USE.

The bottom shelf is of glass to show a leak, and is used to write upon.

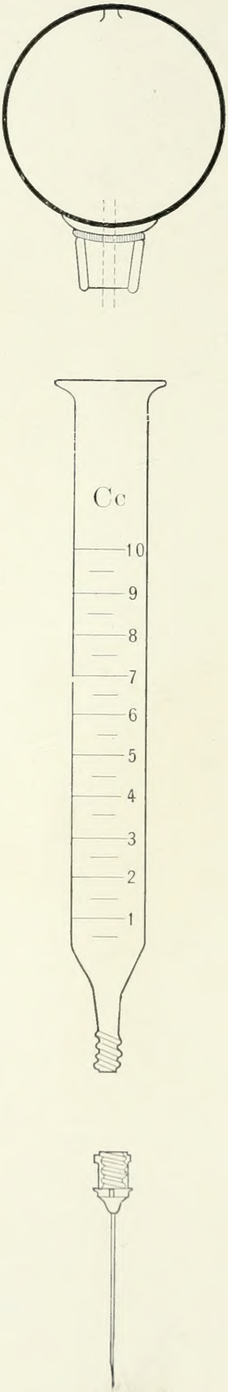

FIG. 2.—SHOWING THE THREE PARTS OF THE SYRINGE, VIZ, THE RUBBER BULB, THE GLASS BARREL, AND THE NEEDLE.

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In citing these bulletins, beginning with No. 8, bibliographers and authors are requested to adopt the following abbreviations: Bull. No. ——, Hyg. Lab., U. S. Pub. Health & Mar.-Hosp. Serv., Wash., pp. ——.

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